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Mycena guldeniana – a new alpine species from Norway

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Abstract — *Mycena guldeniana* (section *Polyadelphia*) is proposed for an agaric found in alpine areas of south Norway that is here described, illustrated and compared with other species of the section. The new species grows on fallen, decaying leaves under *Salix* spp. or occasionally on small twigs in the same habitat and is characterized by its small size, greyish brown pileus, 4-spored basidia, smooth cheilocystidia, prominent, wide terminal cells of the pileipellis hyphae, and clamp connections. Phylogenetic analyses of nucLSU support the taxon within *Mycena* and genetically distinct from the morphologically similar *M. terena*.

Key words — taxonomy, alpine mycology, basidiomycete phylogenetics

Introduction

During a stay at 'Finse Research Centre' in south Norway, August 2005, the first author collected and studied several *Mycena* species. The research centre is situated at approximately 60°30′N on the mountain plateau Hardangervidda 1200 m above sea level. The area lies entirely within the alpine zone and is devoid of trees but in parts is covered with *Salix* shrubs. The research led to the proposal of two new *Mycena* species (Aronsen & Gulden 2007). Two collections were also made of a small *Mycena* that initially was misinterpreted as *M. terena* Aronsen & Maas Geest., a species found on fallen, decaying leaves of *Salix caprea* in the coastal area of South Norway (Aronsen & Maas Geesteranus 1992).

In 2008 the 'Finse research centre' was visited again, and the main investigation was done on the south-facing slope at Nordnut at approximately 1400 m. The slope is formed by basic, phyllitic rocks intermixed with *Salix* shrubs and some calcicolous vegetation (Gulden & Jenssen 1982). On small, partially buried twigs on the ground under the *Salix* shrubs a tiny *Mycena* species was collected. Further investigation showed that not only was it identical with the taxon found in 2005, it clearly differed from *M. terena* and did not match any other known species of *Mycena*.

The same year this taxon was also collected at the base of the mountain Gaustatoppen at approximately 59°51′N at approximately 1150 m. Gaustatoppen (1883 m) is the peak of a 7 km long mountain spine consisting of quarzite, and in the lower slopes there are areas with dense *Salix* shrubs. In these *Salix* shrubs the unknown taxon was found in great numbers together with *M. exilis* Aronsen & Gulden. The taxon is herein proposed as a new species.

Materials & methods

The description is based on six collections from two different years and two different localities including specimens in all stages of age. Preliminary observations and photos were made in daylight in the field, while more thorough observations and descriptions were made the same day in lamp light in the laboratory of the research centre. The dried material was rehydrated in 2% KOH and examined in ammoniated Congo Red and Melzer's reagent using a Nikon light microscope with high resolution, 100x oil objective. For each collection 30 spores were measured. The spore length and width ratios (q) were calculated and the average quotient value (q_{av}) is given in the description. Measurements and drawings were made of basidia, spores, cheilocystidia, pileipellis hyphae, and the stipe cortical layer. Melzer's reagent was used to check amyloidity of spore walls and colour reactions in the lamellar trama. Authors abbreviations follow Index Fungorum (2011), and the collections are deposited in the university museum of Oslo (O).

To assess the phylogenetic position of *M. guldeniana* within *Mycena*, and the relationship of this taxon to the morphologically similar *M. terena*, sequences of the 5′ end of the nuclear large ribosomal subunit gene (nucLSU) spanning domains D1 and D2 were analyzed within a broader sampling of taxa representative of *Mycena* and the tricholomatoid clade sensu Matheny et al. (2006). Two members of *Boletales* were included as outgroup taxa for rooting purposes. Genomic DNA was extracted from dried basidiomes representing each taxon (Table 1) using the E.Z.N.A. Forensic DNA Kit (Omega Bio-Tek, U.S.A.) according to the manufacturer's protocols. PCR protocols followed Perry & Pfister (2007) using primers LROR and LR5 (Moncalvo et al. 2007). Amplified DNA was cleaned with ExoSAP-IT (USB Molecular Biology Reagents and Biochemical, U.S.A.) and sent to Elim Biopharmaceuticals (http://www.elimbiopharm.com/) for cycle sequencing and visualization on an ABI 3700 capillary sequencer (Applied Biosystems, U.S.A.) using the same primers as above. Sequences were edited and assembled using Sequencher 4.0 (Gene Codes Corp., U.S.A.). The resulting data matrix was aligned manually with MacClade 4 (Maddison & Maddison, 2000). Edited sequences have been deposited in GenBank (Table 1), and the aligned dataset is available via TreeBase (www.treebase.org; S11538).

Maximum likelihood (ML) analyses were conducted in PAUP* version 4 (Swofford 2003) and employed an iterative approach. Starting trees were built via the Neighbor Joining method and used to estimate parameters of the model of sequence evolution. Estimated parameter values were then fixed, and a ML search was conducted. Resulting topologies were then used to re-estimate and fix parameter values. This process was repeated for a total of three iterations to insure the analyses had converged on the most likely topology. Clade support was assessed by non-parametric, maximum likelihood

Table 1. List of sequences included in this study and associated GenBank accession numbers.

Species	Collection	GenBank Accession #
Asterophora lycoperdoides (Bull.) Ditmar	CBS 170.86	AF223190
Boletellus projectellus (Murrill) Singer	MB 03-118	NG_027638
Clitocybe dealbata (Sowerby) Gillet	HC 95.cp3	AF223175
Collybia tuberosa (Bull.) P. Kumm.	TENN53540	NG_027631
Entoloma prunuloides (Fr.) Quél.	TJB4765	AY700180
Mycena clavicularis (Fr.) Gillet	RV87/6	AF042637
Mycena crocata (Schrad.) P. Kumm.	BAYER G 057	AY207241
Mycena galericulata (Scop.) Gray	GLM 45970	AY207251
Mycena guldeniana	**A 36/05**	**JF944831**
Mycena haematopus (Pers.) P. Kumm.	HKI ST 22545	AY207252
Mycena insignis A.H.Sm.	DAOM208539	AF261413
Mycena leaiana (Berk.) Sacc.	DAOM167618	AF261411
Mycena maculata P. Karst	GLM 4597	AY207254
Mycena niveipes (Murrill) Murrill	GLM 45975	AY207242
Mycena olivaceomarginata (Massee) Massee	GLM 45976	AY207255
Mycena plumbea P. Karst.	PBM 2718	DQ470813
Mycena polygramma (Bull.) Gray	GLM 45977	AY207243
Mycena pura (Pers.) P. Kumm.	JM98/136	AF261410
Mycena renati Quél.	GLM 45979	AY207256
Mycena rubromarginata (Fr.) P. Kumm.	GLM 45981	AY207245
Mycena sanguinolenta (Alb. & Schwein.) P. Kumm.	GLM 45982	AY207257
Mycena terena	A 41/09	JF944832
Mycena tintinnabulum (Fr.) Quél.	GLM 4598	AY207258
Mycena viscidocruenta Cleland	DUKE3411	AF261414
Mycena zephirus (Fr.) P. Kumm.	GLM 45984	AY207259
Suillus pictus (Peck) A.H. Sm. & Thiers	MB03-002	AY684154
Termitomyces sp.	ZA164	DQ110875
Tricholoma aestuans (Fr.) Gillet	PBM2494	AY700197

bootstrap analyses (Felsenstein 1985) as implemented in GARLI (Zwickl 2006), and consisted of 1000 replicates with all parameter values estimated by the program. All analyses were performed under a GTR+I+G model of sequence evolution as determined using the Akaike Information Criterion in MrModelTest 2.3 (Nylander 2004). Bayesian

analyses were performed using Metropolis-coupled Markov Chain Monte Carlo (MCMCMC) methods as implemented in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) using the same model as the ML analyses. Analyses consisted of two parallel searches, run for 3 million generations and initiated with random starting trees. Chains were sampled every 300 generations for a total of 10,000 trees per search, sampled from the posterior distribution. Trees sampled prior to the analysis reaching a split deviation frequency of 0.02 were discarded as the burn-in, while the remaining trees were used to calculate the posterior probabilities of the individual clades. Default settings in MrBayes were used to set the incremental heating scheme, chain number, unconstrained branch lengths and uninformative topology priors.

Taxonomy

Mycena guldeniana Aronsen & B.A. Perry, **sp. nov.** FIG. 1–7

MycoBank MB 561558

Basidiomata solitaria vel gregaria. Pileus usque ad 2 mm latus, hemisphericus, conicus vel parabolicus, interdum papillatus, plerumque sulcatus, translucente striatus, pruinosus, glabrescens, pallide brunneus, pallide griseo-brunneus vel obscure griseus, centro obscurior. Caro tenuis, pallida, odore nullo. Lamellae 4–8 stipitem attingentes, adnatae vel late adnatae, pallide brunneae, pallide griseae vel albae. Stipes usque 30 × 0.2 mm, brunneo-griseus vel griseus deorsum albido-pallescens, basi fibrillis albis instructus.

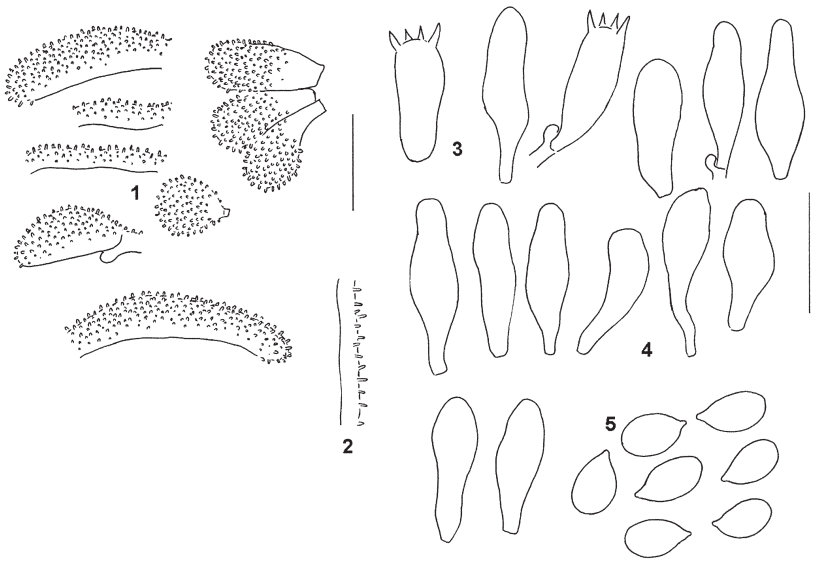
Basidia 18–33 × 7–11 µm, clavata, (2–)4-spora, fibulata. Sporae 7.5–8.7–10 × 4.8–5.5–6.5 µm, amyloideae. Cheilocystidia 21–40 × 6–11 µm, cylindracea, subclavata, sublageniformia, fibulata, laevia. Pleurocystidia nulla. Hyphae pileipellis 5–10(–17) µm latae, fibulatae, diverticulatae, cellulae terminales usque 15 µm latae, clavatae vel subcylindraceae, diverticulatae. Hyphae stipitis corticales 1–7 µm latae, fibulatae, diverticulatae.

Ad Salicis *folia decisa, in zona alpina.*

Type: NORWAY, Telemark, Tinn, Gaustakneet, UTM(WGS84): MM 8249 3560, altitude ca. 1150 m., 21 Sept. 2008, leg. A. Aronsen A54/08 (**Holotype** O-F300034).

Etymology: The species is named after Prof. Gro Gulden, Oslo, in recognition of her contributions to the knowledge of arctic and alpine fungi.

Pileus 1–2 mm across, hemispherical, then conical to parabolical, flattening to convex, becoming more or less plano-convex with or without a small papilla centrally, occasionally somewhat depressed centrally, pruinose, glabrescent, shallowly sulcate, more or less translucent-striate, pale brown or pale grey-brown or grey to dark grey, sometimes pale grey, the centre dark brown to dark grey. Flesh very thin, whitish. Odour and taste indistinctive. Lamellae 4–8 reaching the stipe, with or without lamellulae, usually well developed and fairly broad, but occasionally only showing as faint ridges, ascending, the edge concave to convex, narrowly adnate to broadly adnate, sometimes decurrent with a very short tooth, pale brown, pale grey to white, the edge pallid. Stipe up to 30 × 0.2 mm, very thin, hollow, equal, terete, firm, pruinose, glabrescent, becoming shiny, curved to flexuous, brownish grey to watery grey, becoming more whitish; sometimes dark grey at the apex in younger specimens; often



Figures 1–5. *Mycena guldeniana* (holotype). 1. Hyphae of the pileipellis with terminal cells. 2. Hypha of the cortical layer of the stipe. 3. Basidia. 4. Cheilocystidia. 5. Spores. Bar = 20 μm.

conspicuously insititious but occasionally attached to the substratum by a whorl of radiating, flexuous, white mycelial fibrils.

Basidia 18–33 × 7–11 μm, clavate, (2- and) 4-spored, with plump sterigmata up to 5 μm long. Spores 7.5–8.7–10 × 4.8–5.5–6.5 μm, q = 1.3–1.9, q_{av} ~ 1.6, broadly ellipsoid to pip-shaped, smooth, amyloid. Cheilocystidia 21–40 × 6–11 μm, occuring mixed with the basidia, cylindrical, subclavate, sublageniform, smooth. Pleurocystidia absent. Lamellar trama dextrinoid, reddish brown in Melzer's reagent. Hyphae of the pileipellis 5–10(–17) μm wide, densely covered with cylindrical, straight excrescences 1–3.5 × 0.5 μm; terminal cells up to 40 × 15 μm, clavate to subcylindrical, covered with short, straight, cylindrical excrescences. Hypodermium of broad, cylindrical to subglobose, smooth cells 15–40 × 13–28 μm. Hyphae of the cortical layer of the stipe 1–7 μm wide, densely covered with cylindrical, sometimes more thorn-like, straight excrescences 1–6 × 0.5–1 μm; terminal cells not typically differentiated but a few observed at the base of the stipe being clavate, 26–35 × 5–10 μm, densely covered with cylindrical excrescences 1–4 × 0.5–1 μm. Clamp connections observed at the septa of some of the basidia, the cheilocystidia, and the hyphae of the pileipellis and the stipe cortex.

Solitary or in small groups on fallen, decaying leaves of *Salix* sp.; occasionally on small, fallen twigs on the ground in dense *Salix* shrubs.

Additional specimens examined — **NORWAY**: **Hordaland**, Ulvik, Finse, Nordnut sør, UTM(WGS84): MN 1667 1903, alt. ca. 1400 m, 27 Aug. 2005, leg. A. Aronsen A 34/05 and A 36/05, GenBank JF944831; 23 Aug. 2008, leg. A. Aronsen A 30/08; **Telemark**, Tinn, Gaustakneet, UTM(WGS84): MM 8249 3560, alt. ca 1150 m, 14 Sept. 2008, leg. A. Aronsen A 44/08; 21 Sept. 2008, leg. A. Aronsen A 63/08.

Discussion

Mycena guldeniana belongs to section *Polyadelphia* Singer ex Maas Geest. (Maas Geesteranus 1986) based on its small size, adnate lamellae, pileipellis and stipitipellis hyphae densely covered with short cylindrical excrescences, absence of pleurocystidia, and occurrence on decaying leaves. The species of this section usually possess cheilocystidia that are densely covered with short, cylindrical excrescences, but Aronsen & Maas Geesteranus (1992) described the new species *M. terena* with smooth cheilocystidia. Another species in the same section known to have more or less smooth cheilocystidia is *M. querciphila* Esteve-Rav. & M. Villarreal (Esteve-Raventós & Villarreal 1997).

The smooth cheilocystidia of *M. guldeniana* suggest a close relationship with both *M. terena* and *M. querciphila*, with *M. terena* seeming the most similar. *Mycena querciphila* differs in the pale olive-yellow to olive-brown colours of pileus, lamellae, and stipe and in having some cheilocystidia sparsely diverticulate by warts or short cylindrical excrescences. Additionally, *M. querciphila* is typically found growing on fallen, decaying leaves of *Quercus ilex* subsp. *ballota*. *Mycena terena*, recorded on fallen decaying *Salix caprea* leaves in two coastal lowland localities in South Norway, has a pale beige or very pale grey pileus that soon turns white, a black stipe apex (when young), somewhat narrower spores, and pileipellis hyphae that lack prominent terminal cells. The differences are shown in Table 2 below.

Table 2. A comparison between *Mycena guldeniana* and *M. terena*.

	Mycena guldeniana	*Mycena terena*
Pileus	pale brown to grey-brown	pale beige or pale grey, soon fading to white
Stipe apex	white to dark grey in young specimens	black in young specimens
Spores	q_{av} ~1.6	q_{av} ~ 1.8
Hyphae of the pileipellis	with prominent, inflated terminal cells	without prominent terminal cells
Habitat	on fallen leaves and twigs under *Salix* spp. in alpine area	on fallen leaves of *Salix caprea* in coastal lowland

Figure 6. *Mycena guldeniana* (holotype).

On 11 Aug. 1979, Gulden & Jenssen (1982) reported an unknown species of *Mycena*, collected at Finse, Nordnut, on a dead leaf. Although their material was 'too scanty for a formal description of a new species,' the description indicates that their unknown species was identical with *M. guldeniana*.

Phylogenetic analyses

The nucLSU dataset comprises 887 aligned positions for 26 ingroup taxa and contains 131 parsimony informative characters. Maximum likelihood analyses converged on a common topology, with each iteration producing trees that did not differ significantly in their likelihood values. The final ML tree recovered is shown in Figure 7 (–*ln*L = 3527.88466). Bayesian analyses reached a split deviation frequency below 0.02 after approximately 880,000 generations, and the first 2930 trees sampled were discarded as the burn-in. All *Mycena* sequences included in the analyses form a well-supported (ML bootstrap = 100, posterior probability = 1.0) lineage, sister to the remaining taxa sampled from the tricholomatoid clade. Although relationships within the *Mycena* lineage are not resolved with significant support, *M. guldeniana* and *M. terena* are

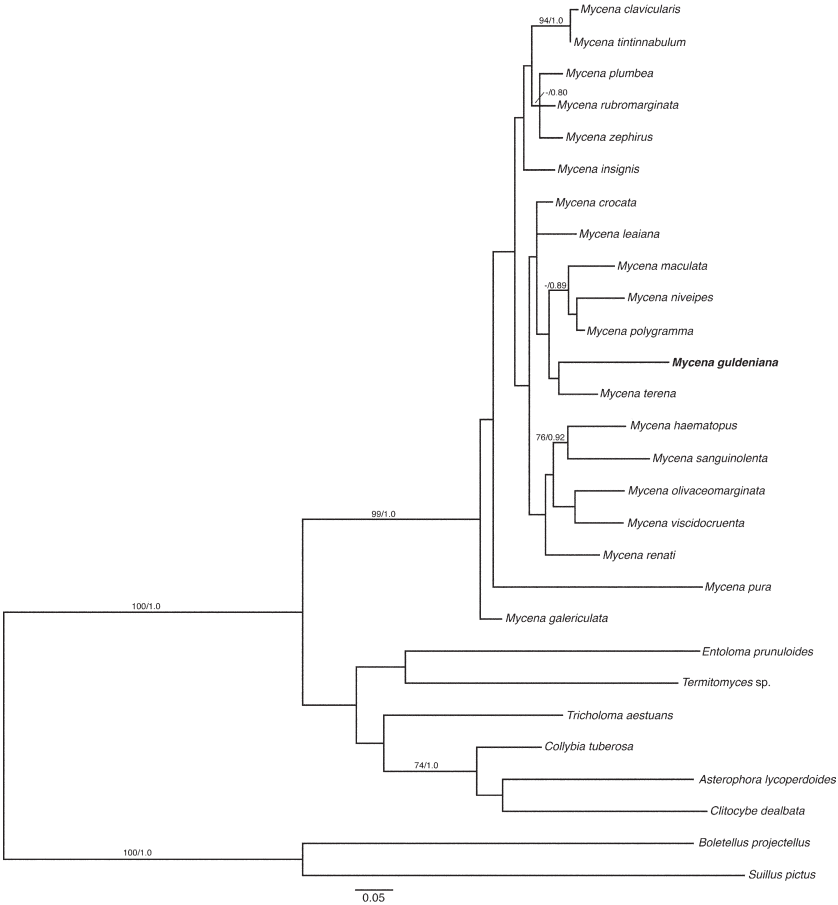


Figure 7. Maximum likelihood topology of *Mycena guldeniana* and other *Mycena* species inferred from nucLSU sequence data (–lnL = 3527.88466). Numbers separated by / represent maximum likelihood bootstrap proportions and bayesian posterior probabilities greater than 70% and 0.70, respectively (– designates a value below 70% / 0.70).

resolved as sister taxa in all topologies recovered by the ML analysis. Although the relationship between these taxa is not well supported by ML bootstrap or bayesian posterior probabilities, these results do support our recognition of *M. terena* and *M. guldeniana* as distinct phylogenetic species. While these two taxa are very similar morphologically, the nucLSU data indicate that genetically they are as distinct from one another as are the majority of other closely related *Mycena* species included in our analyses.

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